

WHAT IS CLAIMED IS:

1. A method for producing an adenovirus comprising:
 - a) growing host cells in media at a low perfusion rate;
 - 5 b) infecting said host cells with an adenovirus;
 - c) harvesting and lysing said host cells to produce a crude cell lysate;
 - d) concentrating said crude cell lysate;
 - e) exchanging buffer of crude cell lysate; and
 - f) reducing the concentration of contaminating nucleic acids in said crude cell
- 10 lysate.
2. The method of claim 1, further comprising isolating an adenoviral particle from said cell lysate using chromatography.
- 15 3. The method of claim 1, wherein the glucose concentration in said media is maintained between about 0.7 and about 1.7g/L.
4. The method of claim 1, wherein said exchanging buffer involves a diafiltration step.
- 20 5. The method of claim 1, wherein said adenovirus comprises an adenoviral vector encoding an exogenous gene construct.

6. The method of claim 5, wherein said gene construct is operatively³ linked to a promoter.
7. The method of claim 6, wherein said promoter is SV40 IE, RSV LTR, β -actin, CMV IE, adenovirus major late, polyoma F9-1, or tyrosinase.
8. The method of claim 1, wherein said adenovirus is a replication-incompetent adenovirus.
9. The method of claim 8, wherein the adenovirus is lacking at least a portion of the E1-region.
10. The method of claim 9, wherein the adenovirus is lacking at least a portion of the E1A and/or E1B region.
11. The method of claim 1, wherein said host cells are capable of complementing replication.
12. The method of claim 1, wherein said host cells are 293 cells.
13. The method of claim 5, wherein said exogenous gene construct encodes a therapeutic gene.

14. The method of claim 13, wherein said therapeutic gene encodes antisense *ras*,
antisense *myc*, Rb, CFTR, p16, p21, p27, p57, C-CAM, APC, CTS-1, scFV *ras*,
DCC, NF-1, NF-2, WT-1, MEN-I, MEN-II, BRCA1, VHL, FCC, MCC, BRCA2,
5 thymidine kinase or p53. *None in list*
15. The method of claim 14, wherein said therapeutic gene encodes p53.
16. The method of claim 1, wherein said cells are harvested and lysed *ex situ* using a
10 hypotonic solution, hypertonic solution, freeze-thaw, sonication, impinging jet,
microfluidization or a detergent.
17. The method of claim 1, wherein said cells are harvested and lysed *in situ* using a
hypotonic solution, hypertonic solution, or a detergent.
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18. The method of claim 17, wherein said cells are lysed and harvested using
detergent.
19. The method of claim 18, wherein said detergent is Thesit®, NP-40®, Tween-20®,
20 Brij-58®, Triton X®-100 or octyl glucoside.
20. The method of claim 1, wherein said lysis is achieved through autolysis of
infected cells.

21. The method of claim 1, wherein said cell lysate is treated with Benzonase®, or Pulmozyme®.
- 5 22. The method of claim 2, wherein said isolating consists essentially of a single chromatography step.
23. The method of claim 22, wherein said chromatography step is ion exchange chromatography.
- 10 24. The method of claim 23, wherein said ion exchange chromatography is anion exchange chromatography.
25. The method of claim 24, wherein said anion exchange chromatography utilizes
15 DEAE, TMAE, QAE, or PEI.
26. The method of claim 24, wherein said anion exchange chromatography utilizes Toyopearl Super Q 650M, MonoQ, or Fractogel TMAE.
- 20 27. The method of claim 24, wherein said ion exchange chromatography is carried out at a pH range of between about 7.0 and about 10.0.

28. The method of claim 1, further comprising a concentration step employing membrane filtration.

29. The method of claim, 28, wherein said filtration is tangential flow filtration.

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30. The method of claim, 28, wherein said filtration utilizes a 100 to 300K NMWC, regenerated cellulose, or polyether sulfone membrane.

31. An adenovirus produced according to a process comprising the steps of:

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a) growing host cells in media at a low perfusion rate;

b) infecting said host cells with an adenovirus;

c) harvesting and lysing said host cells to produce a crude cell lysate;

d) concentrating said crude cell lysate;

e) exchanging buffer of crude cell lysate; and

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f) reducing the concentration of contaminating nucleic acids in said crude cell lysate.

32. The adenovirus of claim 31, further comprising an adenoviral particle isolated from said cell lysate using chromatography.

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33. The adenovirus of claim 31, wherein the glucose concentration in said media is maintained between about 0.7 and about 1.7g/L.

34. The adenovirus of claim 31, wherein said exchanging buffer involves a diafiltration step.
- 5 35. The adenovirus of claim 31, wherein adenovirus comprises an adenoviral vector encoding an exogenous gene construct.
36. The adenovirus of claim 31, wherein said gene construct is operatively linked to a promoter.
- 10 37. The adenovirus of claim 31, wherein said adenovirus is a replication-incompetent adenovirus.
38. The adenovirus of claim 37, wherein said adenovirus is lacking at least a portion of the E1-region.
- 15 39. The adenovirus of claim 31, wherein the adenovirus is lacking at least a portion of the E1A and/or E1B region.
- 20 40. The adenovirus of claim 31, wherein said host cells are capable of complementing replication.

41. The adenovirus of claim 31, wherein said host cells are 293 cells.

42. The adenovirus of claim 31, wherein said exogenous gene construct encodes a therapeutic gene.

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43. The adenovirus of claim 42, wherein said therapeutic gene encodes antisense *ras*, antisense *myc*, Rb, CFTR, p16, p21, p27, p57, C-CAM, APC, CTS-1, scFV *ras*, DCC, NF-1, NF-2, WT-1, MEN-I, MEN-II, BRCA1, VHL, FCC, MCC, BRAC2, thymidine kinase or p53.

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44. The adenovirus of claim 43, wherein said therapeutic gene is p53.

45. The adenovirus of claim 36, wherein said promoter is SV40 IE, RSV LTR, β -actin or CMV IE, adenovirus major late, polyoma F9-1, or tyrosinase.

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46. The adenovirus of claim 31, wherein said cells are harvested and lysed *ex situ* using a hypotonic solution, hypertonic solution, freeze-thaw, sonication, impinging jet, microfluidization or a detergent.

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47. The adenovirus of claim 31, wherein said cells are harvested and lysed *in situ* using a hypotonic solution, hypertonic solution, or a detergent.

48. The adenovirus of claim 31, wherein said cells are harvested using centrifugation.
49. The adenovirus of claim 31, wherein said cells are lysed and harvested using detergent.
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50. The adenovirus of claim 49, wherein said detergent is Thesit®, NP-40®, Tween-20®, Brij-58®, Triton X-100® or octyl glucoside.
51. The adenovirus of claim 31, wherein said cell lysate is treated with Benzonase®,
10 or Pulmozyme®.
52. The adenovirus of claim 32, wherein said chromatography consists essentially of a single chromatography step.
- 15 53. The adenovirus of claim 52, wherein said chromatography step is ion exchange chromatography.
54. The adenovirus of claim 53, wherein said ion exchange chromatography is anion exchange chromatography.
- 20 55. The adenovirus of claim 54, wherein said anion exchange chromatography utilizes DEAE, TMAE, QAE, or PEI.

56. The adenovirus of claim 54, wherein said anion exchange chromatography utilizes Toyopearl Super Q 650M, MonoQ, or Fractogel TMAE.
- 5 57. The adenovirus of claim 54, wherein said ion exchange chromatography is carried out a pH range of between about 7.0 and about 10.0.
58. The adenovirus of claim 31, further comprising a concentration step employing membrane filtration.
- 10 59. The adenovirus of claim, 58, wherein said filtration is tangential flow filtration.
60. The adenovirus of claim, 59, wherein said filtration utilizes a 100 to 300K NMWC, regenerated cellulose, or polyether sulfone membrane.
- 15 61. A method for the purification of an adenovirus comprising:
- =43 +
- a) growing host cells;
- b) infecting said host cells with an adenovirus;
- c) harvesting and lysing said host cells by contacting said cells with a detergent to
- 20 produce a crude cell lysate;
- d) concentrating said crude cell lysate;
- e) exchanging buffer of crude cell lysate; and

f) reducing the concentration of contaminating nucleic acids in said crude cell lysate.

5 62. The method of claim 61, further comprising isolating an adenoviral particle from said lysate using chromatography.

63. The method of claim 61, wherein said host cells are grown in media wherein a glucose concentration is maintained between about 0.7 and about 1.7g/L.

10 64. The method of claim 61, wherein said exchanging buffer involves a diafiltration step.

65. The method of claim 61, wherein said detergent is Thesit®, NP-40®, Tween-20®, Brij-58®, Triton X-100® or octyl glucoside.

15 66. The method of claim 65, wherein said detergent is present in the lysis solution at a concentration of about 1% (w/v).

20 67. The method of claim 61, wherein said isolating consists essentially of a single chromatography step.

68. The method of claim 62, wherein said chromatography step is ion exchange chromatography.

69. An adenovirus produced according to a process comprising the steps of: =57
- a) growing host cells;
 - b) infecting said host cells with an adenovirus;
 - 5 c) harvesting and lysing said host cells by contacting said cells with a detergent to produce a crude cell lysate;
 - d) concentrating said crude cell lysate;
 - e) exchanging buffer of crude cell lysate; and
 - f) reducing the concentration of contaminating nucleic acids in said crude cell
- 10 lysate.
70. The adenovirus of claim 69, further comprising an adenoviral particle isolated from said lysate using chromatography. ohf
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71. The adenovirus of claim 69, wherein said host cells are grown in media
- 15 comprising a glucose concentration of less than 1g/L.
72. The adenovirus of claim 69, wherein said detergent is Thesit®, NP-40®, Tween-20®, Brij-58®, Triton X-100® or octyl glucoside.
- 20 73. The adenovirus of claim 72, said detergent is present in the lysis solution at a concentration of about 1% (w/v).

74. The adenovirus of claim 70, wherein said isolating consists essentially of a single chromatography step.

75. The adenovirus of claim 70, wherein said chromatography step is ion exchange chromatography.

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